

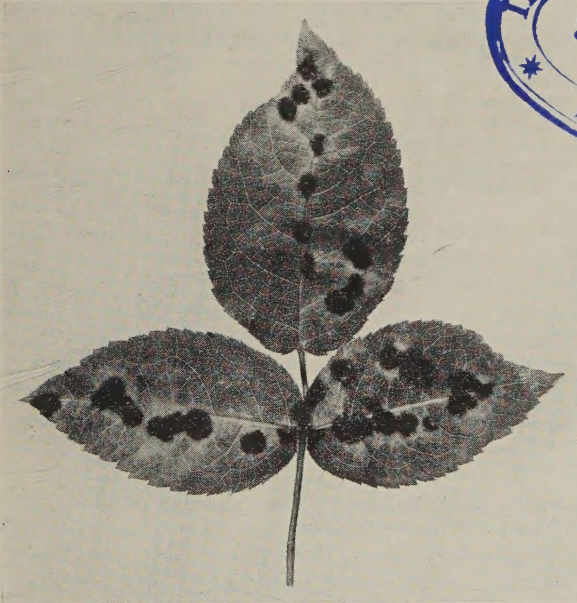
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The Black-Spot Disease of Roses, and Its Control under Greenhouse Conditions

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THE BLACK-SPOT DISEASE OF ROSES, AND ITS CONTROL UNDER GREENHOUSE CONDITIONS¹

E. W. LYLE

Black-spot has long been recognized as one of the most serious diseases of the rose, both of plants grown under glass and of those grown in the open. Symptoms of the disease appear as spots or blotches mostly on the leaves, impairing the appearance of the foliage and causing premature defoliation. While the disease is generally present in rose cultures, epiphytotics with resulting severe damage are frequent. At such times spotting and defoliation may be so serious as to severely affect the vitality of the plants.

VARIETAL SUSCEPTIBILITY

General observation has shown that certain kinds of greenhouse roses are more severely affected than are others. Some varieties, such as Pernet, Premier Supreme, Hollywood, Rapture, Briarcliff, Templar, Sweet Adeline, Joanna Hill, and Better Times, are commonly attacked by black-spot. Other varieties, such as Double White Killarney, Talisman, Mrs. Franklin D. Roosevelt, and Souvenir, are usually less affected.

An experimental test with potted plants of the varieties Signet, Token, Better Times, Chieftain, Pernet, Premier Supreme, Joanna Hill, Double White Killarney, and Talisman indicated that these varieties are about equally susceptible when conditions are uniformly favorable for infection. Dodge (1931) found that Red Radiance, a reputedly fairly resistant variety, could be infected with black-spot just about as easily as could Felicity, Padre, Mrs. Henry Bowles, and Charles K. Douglas, varieties said to be susceptible to the disease. The conclusion is reached that all varieties of roses may be affected by black-spot when environmental influences favor infection. The differences among varieties under ordinary greenhouse conditions may be due to variations in transpiration or differences in the leaf surface with respect to wettability and rapidity of drying.

THE DISEASE

HISTORY AND RANGE

Black-spot in greenhouses has existed probably as long as roses have been forced under glass. In the United States the first published reference is probably that by Scribner (1888). Later, Maynard (1889) and Humphrey (1889) reported on the injury to greenhouse roses caused by

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black-spot. Subsequently the disease has been more frequently recognized, and at the present time there are few growers of greenhouse roses who have not observed and experienced trouble with it.

ECONOMIC IMPORTANCE

Black-spot has often been classed as the most important disease of roses. This is true of both greenhouse and outdoor roses. Plants which have been partially defoliated by black-spot are weakened, and the flowers produced do not equal in quantity or quality those borne on plants free from the disease.

The effect of black-spot in decreasing the number of leaves was shown by a test in which the disease was controlled in a part of a greenhouse containing the varieties Premier Supreme and Rapture. Where the disease was controlled there were as many as 20 per cent more leaves after fourteen weeks than on the untreated plants. Besides the effect on number of leaves, the average leaf-area also is influenced. The loss of leaves and the failure of those remaining to reach maximum size causes a reduction in total photosynthetic activity, affecting the production of plant food, decreasing the vigor of the plant, and lowering the number and quality of blossoms.

The action of black-spot becomes more serious as it persists in a rose house through an entire season. Two or more successive defoliations by black-spot weaken the plants and may make them so inferior that replanting becomes necessary. If the disease has been particularly serious, the entire house may have to be replanted. If the injury has been slight, replacing only the weakened or dead plants may suffice. In a house of 10,000 one-year-old plants of the variety Sweet Adeline where black-spot was severe, it was found that 10 per cent of the plants were either dead or so weakened that they were decidedly inferior. A house in which the disease is prevalent may have to be replanted after one or two years, while otherwise this expensive operation may be delayed until the fifth or the sixth year.

Heavy pruning of the plants in midseason has been resorted to by some growers in the hope of eliminating further trouble from the disease. Such midseason pruning results in the loss of about three months of flower production during the best marketing period, causing substantial monetary loss.

Failures in grafting may sometimes be partially attributed to black-spot. When the leaves of the scion are spotted or become spotted in the course of grafting, such leaves may drop early and result in weakened grafts and then failure to develop properly. Losses in cutting beds are sometimes similarly due to premature loss of leaves by black-spot.

The value of cut roses is based on the length and strength of stem as well as upon flower perfection. Where black-spot has caused weakened growth and smaller development, the market value is decreased. The commercial grower is also continually confronted by the demand for clean foliage. Any defoliation, spotting, or other disfiguration detracts materially from the sales value of such flowers. The economic importance is better understood when the value of the rose industry itself is cited.

The horticultural census (Austin, 1932) gives an estimate of \$23,708,197

as the total receipts from sales of cut and pot roses grown under glass in the United States during 1929. This value was obtained from reports of 1220 establishments growing roses for cut flowers, 951 producing potted roses, and 123 propagating roses for florists. The area under glass devoted to the production of roses for cut flowers was 34,129,855 square feet, or approximately 783.5 acres, for the 1220 establishments reporting.

SYMPTOMATOLOGY

Morphologic symptoms

The usual morphologic symptoms are the more-or-less circular black lesions or spots on the leaf blades. Occasionally, similar symptoms occur also on the stems, the leaf petioles and stipules, and the flower receptacles and sepals.

On the leaf blades

Black necrotic areas develop on the upper surface or on both the upper and the lower surface of the leaves, depending on where the fungous infection has originated. Massey and Westcott (1929) were the first to note that infection may take place on either surface of the leaf. If penetration occurs through the upper epidermis, the spot usually is apparent only on the upper surface. Only occasionally does the fungus grow through to the lower side while the leaf remains attached to the plant. If, however, invasion is through the lower epidermis, the fungus grows through to the upper surface almost immediately, and symptoms and signs appear on both surfaces of the leaf. (Figures 1 and 2, pages 6 and 7.)

As the fungus develops, the spots increase in diameter at the rate of about 1 millimeter each week, depending on the age of the leaves and on environmental factors. The growth is faster in young or active leaves than in old leaves. The spots vary somewhat in appearance. Some have characteristic radiating margins; others do not show such feathery borders. New lesions, particularly, appear as fine networks in the leaf tissue, each lesion when first visible being less than 1 millimeter in diameter. These lesions increase in size, become definite spots, and after several weeks may reach a diameter of 1 centimeter or more. Frequently spots coalesce and give irregularly shaped lesions of larger size.

Leaves affected with black-spot often show symptoms of yellowing. This yellowing may appear as a zone around the black-spot area, or it may involve the entire leaflet.

Premature abscission of leaves is one of the most pronounced characteristics of the black-spot disease. That plants affected by this disease generally have a partly defoliated appearance has been mentioned by Massey (1918).

On the petioles and stipules

Black necrotic areas may be formed on stipules and petiole, and with their development girdle the petiole. Parts thus affected frequently do not absciss prematurely, as is characteristic of leaf blades bearing black-spot, but may be retained and serve as the source of inoculum for further spread of the pathogene. Such lesions are not conspicuous and often

pass unobserved. In a house of 10,000 roses of the variety Briarcliff, on which the disease was controlled after the plants had become badly infected, it was noted that lesions still persisted on some of the petioles and stipules although there were very few attached leaves with lesions on the leaf blades.



A

B

FIGURE 1. UPPER SURFACE OF PERNET ROSE LEAVES FIFTEEN DAYS AFTER INOCULATION WITH THE BLACK-SPOT FUNGUS

A, Leaves inoculated on lower surface. B, Leaves inoculated on upper surface

On the stems

The development of black-spot on the stems is rare in comparison with leaf lesions. The black lesions do occur, however, on the younger and softer wood of certain varieties. The greenhouse varieties Pernet and Sweet Adeline have been found to exhibit stem infections more frequently than have some of the other varieties.

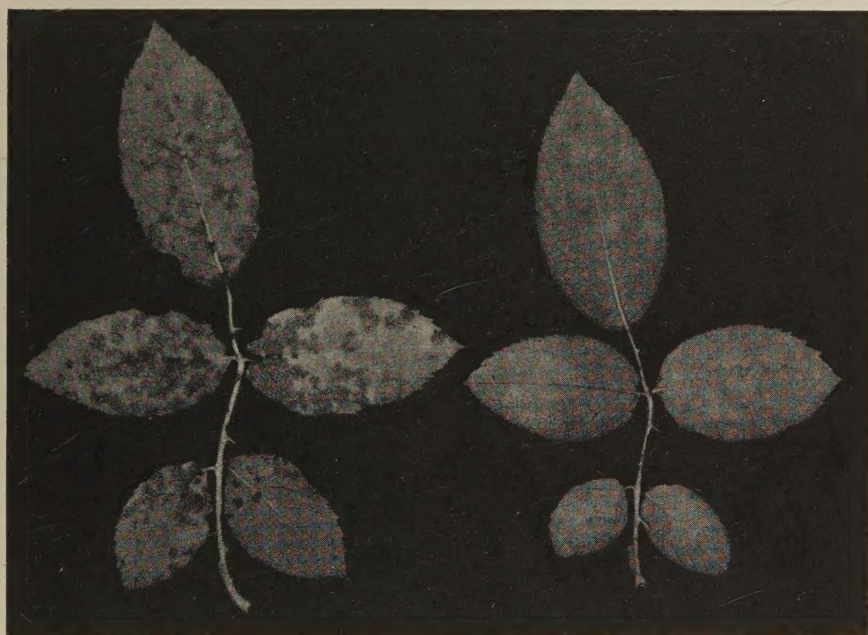
On the flower receptacles and sepals

Black-spot has been noted on receptacles and sepals of greenhouse-grown roses of the variety Pernet. The symptoms are the necrotic black areas characteristic of the disease on other parts of the plant.

Signs

Signs of the disease are the small mounds of conidia which pile up in the acervuli. These spore mounds appear as tiny dark specks within the

blackened areas, and are most noticeable close to the margins of those areas. Observations of black-spot on outdoor roses showed that the number of spore mounds within a single lesion or in a certain area of a lesion varies with the leaf age, the size of the spots, and the environmental conditions under which the mounds develop. Wide variation of the



A

B

FIGURE 2. LOWER SURFACE OF LEAVES SHOWN IN FIGURE 1

A, Leaves inoculated on lower surface. B, Leaves inoculated on upper surface.

number of spore mounds occurred in individual varieties of roses, so that no separation of varietal differences could be made. The most consistent variation, however, was in the number of spore mounds which occurred on the upper surface of the leaves in comparison with those on the lower surface, about 50 per cent more being formed on the upper surface than on the lower.

The acervuli either are restricted to the upper surface of the leaf, or else occur on both surfaces, depending on the manner of infection. As stated on page 5, only in the case of infection through the lower surface are acervuli ordinarily found on both sides of the leaf.

The fact that the spore mounds are mostly apparent in the outer margins of the lesion may be due to the failure of the centrally located acervuli to generate new spores sufficiently to form macroscopic masses after the conidia have once been washed away by rains or by watering. The outer spore mounds may also represent new acervuli which have not been subjected to rain or splashing water after their formation. Further-

more, staling products of the fungus or degeneration of the cells of the suspect may account for a lack of spore mounds toward the center in the older lesions.

Although these studies concerning number and location of spore mounds were made with outdoor leaf material, it is believed that they give a general idea of signs which occur under greenhouse conditions.

LIFE HISTORY OF THE PATHOGENE

The fungus causing black-spot of roses is *Diplocarpon rosae* Wolf. The perfect stage was found and determined by Wolf in 1911, when he examined diseased leaves which had been overwintered outdoors (Wolf, 1912).

Only secondary cycles of the pathogene are common in greenhouses, and, as yet, no evidence has been obtained of the ascigerous stage there. The conidial form is either generally present in varying degrees of severity, or brought into the houses on the roses during replanting.

The fungus frequently is carried over from one season to another in the leaves which are left when the plants are pruned. It has been observed that even when the plants are given a heavy or a "full" prune, some attached leaves are left. A few black-spotted leaves overlooked in this way are sufficient to supply inoculum and cause infection of the subsequent new growth.

With young grafted stock, the pathogene is established if the foliage of the scion wood is infected or if any viable spores of the fungus are present. The conditions of moisture and temperature in the grafting case are optimum for the development and spread of black-spot. Once the grafted plants become diseased, the trouble is certain to be carried into the greenhouse at planting time.

INOCULATION

In greenhouses, dissemination of conidia of *D. rosae* is accomplished mainly by splashing water when the plants are being syringed or watered. The conidia are loosened from the acervuli and are carried by the water to the infection courts. Tests to determine the possibility of dissemination by air currents have given only negative results. Spore traps through which air has been drawn after passing over black-spotted leaves have yielded no conidia of *D. rosae*. Diseased and healthy plants have been maintained adjacent to one another in greenhouses without the spreading of black-spot, merely by preventing water from being splashed from the diseased to the healthy plants.

An indication of the amount of inoculum available for inoculation was obtained by counting the number of spore mounds per unit of black-spot area. Counts of conidia from single spore mounds and from individual lesions of measured size were made also. Infected leaves from outdoor roses were the principal source of these data. Using a binocular microscope and 60x magnification, observations were made of twenty spots from eight outdoor hybrid-tea-rose varieties where spotting occurred on the upper side of the leaves. The average number of spore mounds per unit of black-spot area 4 millimeters in diameter was found to be $74.35 \pm$

3.28 (standard error).² This is comparable to 592 spore mounds per square centimeter of black-spot area on the upper surface of leaves. Examination of twenty-nine spots on the lower surface of leaves gave an average of 395 spore mounds per square centimeter of black-spot area.

Counts of the number of conidia in the largest spore mounds were made by picking off individual mounds with the aid of a binocular microscope and suspending the spores in a measured volume of water. From suitable dilutions of the suspension the number of conidia per drop was determined with the use of a microscope. Either aniline blue or cotton blue was added to the drops to stain the spores and facilitate counting. Replications for statistical treatment were obtained by examining three or four drops of the same spore suspension. Eighteen spore mounds from fresh leaves of eight varieties of outdoor hybrid-tea roses were examined and were found to have an average of 4247 ± 337 conidia each.

An estimate of the number of conidia per black-spot on a leaf was determined by similar dilution methods, the conidia from each leaf lesion being washed in a definite quantity of water. Counts from twelve black-spots of outdoor hybrid-tea roses gave an average of $32,060 \pm 1,566$ conidia per black-spot, the lesions averaging 6.29 millimeters in diameter.

The very great number of conidia available for dissemination and inoculation is thus evident in the case of outdoor roses. Counts of conidia from leaf lesions on greenhouse roses would probably yield similar results.

Because of the development of leaf-spots and of the fungous fruiting structures, new conidia are formed continually as long as conditions are suitable. In one test, excised black-spotted leaves from greenhouse roses were washed daily to remove the mature conidia, and each day a new crop of spores was produced for a period of more than a week. The leaves were kept in a moist chamber at room temperature between washings. An estimate of the number of conidia obtained from each day's washing is given in table 1. The leaf used in this test had two black-spots at the

TABLE 1. NUMBER OF CONIDIA OF DIPLOCARPON ROSAE OBTAINED DAILY FROM BLACK-SPOT LESIONS ON AN EXCISED LEAFLET OF A PERNET PLANT UNDER ARTIFICIAL CONDITIONS, SEPTEMBER 1936

Observation	Number of conidia from leaflet	Number and size of lesions
1st day*	616,000 $\pm$ 30,800	2 lesions, each 10 mm in diameter
2d day	84,000 $\pm$ 3,360	2 lesions, each 10 mm in diameter
3d day	64,400 $\pm$ 5,320	2 lesions, each 11 mm in diameter
4th day	106,400 $\pm$ 4,200	New infections, entire leaflet
5th day	162,400 $\pm$ 3,620	New infections, entire leaflet
6th day	336,000 $\pm$ 11,760	New infections, entire leaflet
7th day	851,200 $\pm$ 25,760	New infections, entire leaflet
8th day	375,200 $\pm$ 17,360	New infections, entire leaflet

*The first record is the number of conidia accumulated from the time of inoculation (August 24 to September 16, 1936), the leaflet not having been washed with water during that period.

²The standard error of the mean was determined by applying the formula

$$\text{S.E.} = \sqrt{\frac{\Sigma A^2 - \frac{(\Sigma A)^2}{n}}{n(n-1)}}$$

in which "A" is the value of single observations and "n" is the number of observations. The standard error is referred to wherever the sign $\pm$ appears.

outset, inoculation having occurred twenty-three days before the leaf was picked and washed the first time.

The total number of conidia obtained in the last seven days of the test was $1,979,600 \pm 34,258$. The number of conidia and the standard error in each case were determined by counting the spores in each of four drops of the same spore suspension. The difference in production of conidia for successive days is significant.

In another test the number of conidia produced by a single lesion in an attached leaf was determined at three-day intervals for a period of about six weeks. A leaflet of a potted Pernet plant was inoculated on the lower surface so that a single lesion developed. Beginning fourteen days after inoculation, with the appearance of the first mounds the leaflet was washed in a measured volume of distilled water every three days, and the number of conidia obtained in this manner was determined by microscopic counts of the spores in representative drops of the spore suspension. The plant was maintained under greenhouse conditions with the temperature about 65° F. and the relative humidity averaging 66.2 per cent. The number of conidia and the standard error in each case were determined from counts of spores in each of three drops of the same spore suspension. The data are presented in table 2. These results again indicate the large quantity of inoculum available for the spread of the black-spot pathogene.

TABLE 2. NUMBER OF CONIDIA OF *DIPLOCARPON ROSAE* OBTAINED FROM A SINGLE BLACK-SPOT LESION IN AN ATTACHED LEAFLET OF A PERNET PLANT UNDER GREENHOUSE CONDITIONS, FEBRUARY AND MARCH 1937

Number of days after inoculation	Number of conidia from lesion	Diameter of lesion (millimeters)
14.....	7,840 $\pm$ 1,232	3.0
17.....	3,360 $\pm$ 504	3.5
20.....	8,120 $\pm$ 840	4.5
23.....	26,600 $\pm$ 2,268	6.0
26.....	22,120 $\pm$ 672	6.5
29.....	19,600 $\pm$ 1,260	7.0
32.....	19,320 $\pm$ 252	8.0
35.....	27,440 $\pm$ 2,100	8.5
38.....	14,840 $\pm$ 1,092	8.5
41.....	22,680 $\pm$ 1,344	9.0
44.....	13,160 $\pm$ 1,148	9.8*
47.....	6,720 $\pm$ 700	9.8
50.....	2,800 $\pm$ 420	9.8†
53.....	1,120 $\pm$ 252	9.8‡
56.....	420 $\pm$ 238	9.8§
Total.....	196,140 $\pm$ 4,386	

*Leaflet one-half yellowed.

†Leaflet nine-tenths yellowed and brown.

‡Leaflet nine-tenths brown and dried.

§Leaflet entirely brown, dried, and detached.

INCUBATION

Conditions which favor inoculation, such as syringing and spattering water, also favor immediate germination of the conidia of *D. rosae* and limit the incubation period to a relatively brief time. If the environment is such that the water evaporates quickly from the foliage, the conidia adhere to the surface and develop only if a condition of extremely high relative humidity or contact with water is acquired within about two weeks time. The conidia lose their vitality and become inactive if dried longer (table 8, page 16).

The temperature relation is scarcely a limiting factor. The conidia germinate over as wide a range of temperatures as would normally occur on the rose foliage in the greenhouse.

Under favorable conditions, conidia germinate within about nine hours. A relative humidity of at least 97 per cent or contact with water is necessary, with the optimum between 99 per cent and immersion in water (table 4, page 12). The optimum temperature for germination is about 26° C., the minimum is about 15°, and the maximum is approximately 33° (table 3).

Conidia do not need contact with rose tissue in order to germinate, and many of the germination tests were made with the spores placed on glass slides. To determine the effect of temperature on germination, conidia from an infected leaf were suspended in distilled water. Drops of the suspension were placed on clean glass slides, and as much water as possible was removed with filter paper, the spores being left on the slides. The glass slides were then dried in air for fifteen minutes before being placed in inverted petri dishes sealed with water and used as moist chambers. This system was used in preference to leaving the conidia suspended in water, since preliminary tests had given more uniform results in this way. The petri dishes were then placed in chambers at the designated temperatures for forty-seven to fifty-three hours before germination counts were made. The average count of three spore mounts is given for each temperature in table 3.

TABLE 3. INFLUENCE OF TEMPERATURE ON GERMINATION OF CONIDIA OF *DIPLO-CARPON ROSAE* ON GLASS SLIDES

Temperature (centigrade)	Average per cent germination
32.7°	0.7
29.0°	26.5
26.2°	52.6
25.3°	44.0
21.8°	2.6
18.4°	0.8
13.8°	0.5

To determine the percentage relative humidity required for conidial germination, the development of spores on glass slides was again used. The spore suspension was prepared by washing leaf lesions in distilled water (except as noted), and drops of the suspension were placed on clean glass slides. In all the tests, except in the checks (in which the spores were left suspended in water), the water was removed with filter paper and the slides were allowed to dry in air for fifteen minutes before being placed in humidity-control chambers. The humidity-control chambers were sealed stender dishes containing sulfuric-acid solutions to control the relative humidity and a glass rack to hold the slides. The concentrations of sulfuric acid were determined by specific-gravity measurements (Stevens, 1916; Wilson, 1921). In each spore mound 250 or more conidia were examined for germination counts, and each percentage listed in table 4 is an average of three or four mounts, as indicated, for the same spore suspension for each relative humidity. The checks in each case were drops of the spore suspension maintained on glass slides without drying.

TABLE 4. RELATION OF RELATIVE HUMIDITY TO GERMINATION OF CONIDIA OF DIPLO-CARPON ROSAE ON GLASS SLIDES

Test	Number of counts averaged	Medium	Relative humidity				
			Check	100%	99%	98%	97%
			Per cent germination				
1	4	Distilled water	17.1	35.5	9.8	1.9	0.0
2	4	Distilled water	22.2	21.8	11.1	2.2	0.3
3	4	Distilled water	10.5	21.6		0.4	0.0
4	4	Distilled water	21.3	36.4		...	0.0
5	4	Distilled water		41.5	17.5	5.0	0.1
6	4	Distilled water	2.4	34.8	12.6	0.1	0.0
7	3	Distilled water	2.4	35.3	5.5	0.0	0.0
	3	Distilled water	3.1	27.2	11.2	0.3	0.0
8	3	Distilled water, spores scraped loose*	11.4	29.3	22.0	0.9	0.0
	3	Distilled water, spores washed loose†	33.4	28.1	23.7	2.2	0.1
9	3	Tap water, spores washed loose†	8.2	21.8		0.2	0.0
	3	Distilled water, spores washed loose†	60.2	43.3	41.1	4.0	0.0
Average germination.....			17.5	31.5	17.2	1.6	0.04

*"Spores scraped loose" indicates that the spore mounds were removed from the leaf lesions with the aid of a scalpel and were then placed in water.

†"Spores washed loose" indicates that the leaf lesions were shaken in a test tube of water to remove and collect the spores.

High humidity alone is not sufficient to cause germination of conidia of *D. rosae*. Tests indicate that wetting of the spores also is necessary before development can occur. Conidia were picked off leaf lesions as spore mounds and placed on glass slides in an atmosphere of approximately 100 per cent relative humidity (over distilled water in a water-sealed moist chamber) for forty-eight hours. The spores did not germinate unless they were first wet with water. If contacted with water first, even for a brief period, and then placed in a chamber with 100 per cent relative humidity, the conidia germinated readily. The data presented in table 5 are averages of germination counts for three spore mounds in each case.

TABLE 5. RESULTS OF EXPERIMENTS TO DETERMINE THE NECESSITY OF PREWETTING FOR SPORE GERMINATION

Test	Treatment previous to placing in 100 per cent relative humidity*	Average per cent germination
1	Spore mounds placed on glass slide; drop of distilled water added; drop not dried	40.2
2	Spore mounds placed on glass slide; no water added	0.0
3	Spore mounds placed on glass slide; drop of distilled water added, then removed immediately with filter paper; dried in air for 15 minutes	0.6
4	Spore mounds stirred in drop of distilled water; water taken up immediately with filter paper; slide dried in air 15 minutes	35.5
5	Spore mounds stirred in drop of distilled water; after 5 minutes the water taken up with filter paper; slide dried in air 15 minutes	32.3

*Assumed value for relative humidity based on concentration of sulfuric acid used. (See page 11.)

The data in table 5 show that, although the conidia of the black-spot fungus must be prewet before they will germinate, the length of time of contact with water for sufficient wetting is somewhat less than five minutes. If, soon after the prewetting, the spores are placed in a saturated atmosphere, they will germinate readily without further contact with water.

Further studies of the effect of prewetting the conidia on their subsequent germination indicated that, whereas conidia which have not been prewet fail to germinate in a saturated atmosphere, conidia which have been prewet will germinate under such conditions even if they are allowed to dry for as long as forty-eight hours after the prewetting. The percentage of germination, however, was found to decrease as the time of drying was prolonged.

The spores, as in the previous test, were taken from leaf lesions without wetting, and were either placed on a dry glass slide or stirred into drops of distilled water on the slide. Where they were suspended in water, the water was removed within five minutes with the aid of filter paper, and the slides were allowed to air-dry for different periods (table 6) at a temperature of about 70° F. and a relative humidity of about 30 per cent. The slides were then placed in a water-sealed moist chamber (77° F. and approximately 100 per cent relative humidity) for forty-eight hours, when germination counts were made. Each percentage given is the average for three spore mounts treated in the same manner, with more than 250 conidia observed in each mount.

TABLE 6. RESULTS OF EXPERIMENTS TO DETERMINE THE EFFECT ON GERMINATION OF DRYING AFTER PREWETTING THE CONIDIA

Test	Treatment previous to placing in 100 per cent relative humidity*	Average per cent germination	
		Series 1	Series 2
1	Not prewet	0.0	0.0
2	Prewet; water removed; not dried	56.4	44.9
3	Prewet; water removed; dried 6 hours	40.0	
4	Prewet; water removed; dried 12 hours	37.3	24.8
5	Prewet; water removed; dried 24 hours		20.4†
6	Prewet; water removed; dried 48 hours		15.0

*Assumed value for relative humidity based on concentration of sulfuric acid used. (See page 11.)

†Average germination of only two conidial mounts instead of three.

This experiment is significant in that it shows that conidia which have been prewet do not require precipitated moisture for germination although they may have been dried for several hours.

The conidia lose their infectibility within a week or two under ordinary conditions (table 8, page 16). Frequently, though, the water which has been the agent of inoculation also favors spore germination and infection. The conidia may be in contact with the water long enough to germinate and infect, or, with the evaporation of the water, the relative humidity may be sufficiently high to result in germination and infection.

INFECTION

With greenhouse roses, leaf lesions become visible within five to ten days after initial infection. Mature acervuli are produced within a day or two after the lesions have become macroscopically apparent. A test of inoculation on nine varieties of greenhouse roses resulted in infection in all cases, the symptoms appearing a day or two later on some varieties than on others. However, as much difference as that may be due to the age of the leaves inoculated, the younger leaves showing symptoms more

quickly than do the older leaves. Also, some variation was found to exist between infections initiated on the upper surface and those started on the lower surface of the leaves. With inoculation of the lower surface, from 92 to 99 per cent infection was obtained. With inoculation of the upper surface of leaves of the same varieties, infection varied from 56 to 100 per cent. It is concluded that conditions on the under side of rose leaves are somewhat more conducive to infection than those on the upper surface. Since the stomata are limited to the lower surface, the factor of relative humidity may be involved. Differences in the cuticle on the two surfaces also may be involved as factors in penetration and infection.

Dodge (1931) showed that the fungus does not gain entrance through the stomata.

SAPROGENESIS

The phase of the life cycle during which the pathogene is not in direct association with the living suspect is probably unimportant in the occurrence of the disease in greenhouses. The black-spot lesions on the foliage and other parts of the plants provide a continuous supply of inoculum for perpetuating the disease. Other sources of inoculum are at most only of minor importance to the continuance of black-spot on plants under glass.

SECONDARY CYCLES

One cycle may follow another after a period of a week or ten days if conditions are favorable. The conidia of *D. rosae* produced in one cycle are capable of germination and infection immediately after dissemination. The frequency of cycles in the greenhouse depends entirely on the factors causing dissemination and germination of the spores, being correlated often with times of syringing with water when the weather is such that the foliage does not dry quickly or when a cloudy period follows soon after the syringing.

EPIPHYTOLOGY

EFFECT OF TEMPERATURE

The greatest effect of temperature on the incidence of black-spot is its inverse relation to atmospheric relative humidity. With high temperature, the relative humidity tends to be low and unfavorable for the initiation of black-spot. Conversely, if the temperature is low, the relative humidity is usually high and conducive to new black-spot infection.

Temperatures tend to be highest at midday, with the relative humidity lowest then; at night the temperatures are generally the lowest and the relative humidity the highest. This relationship is particularly noticeable in the greenhouse during the spring, summer, and fall months, when little or no supplementary heat is supplied. During these months the greatest extremes of temperature occur, affecting the relative humidity the most and causing it to be extremely variable. At this time the greatest number of hours of high relative humidity occurs (table 12, page 19), and conditions are the most suitable for black-spot infection.

EFFECT OF HUMIDITY

One of the main factors influencing the severity of the black-spot disease is relative humidity. Extremely high relative humidities are essential for spore germination, as is pointed out in table 4. Factors favorable for spore germination are correlated with those for infection, and tests made with plants to determine what relative humidity is necessary for infection have shown that infection does not take place unless the relative humidity about the leaf is approximately 92 per cent for more than six hours. The experimental plants were placed in a closed chamber and the humidified air was circulated around them.

These tests with humidified air in motion around the plants are admittedly not comparable with the true conditions in the greenhouse, where, with lack of such air circulation, the relative humidity of the greenhouse atmosphere may be considerably lower than that of the air in close proximity to the leaf surfaces of the plants. Nevertheless, the experiments prove the necessity of a high relative humidity close to the leaf surface before leaf infection by conidia will occur.

The relation of the length of time that conidia are in contact with water (or at an extremely high relative humidity), and the temperature, to the amount of black-spot infection, is shown in table 7. Inoculum for this and subsequent tests consisted of a suspension of conidia prepared by shaking black-spotted leaves in distilled water. The potted rose plants in each test were all inoculated on the upper surface of the leaves, and were placed together in a chamber under conditions favoring infection. At the intervals shown in the table, one plant at a time was removed from the chamber and placed immediately in a room in the greenhouse where the foliage would dry quickly. The plants were fanned for an hour or two until the water had completely evaporated from the foliage. Then they were kept in the greenhouse at normal humidity and temperature. Infection developed only where the foliage after inoculation was prevented from drying for at least six hours. Infection occurred at all the temperatures used.

TABLE 7. RELATION OF LENGTH OF TIME CONIDIA OF *DIPLOCARPON ROSAE* ARE IN CONTACT WITH WATER (OR AT AN EXTREMELY HIGH RELATIVE HUMIDITY), AND TEMPERATURE, TO THE NUMBER OF LEAF INFECTIONS

Length of time foliage was wet (hours)	Temperature (Fahrenheit)			
	50-53°	59-62°	67-69°	74-75°
	Number of lesions per 100 leaflets			
6	..	0	0	0
7	..	8	45	10
8	..	15	59	8
9	..	42	135	36
10	0	54	434	263
11	..	164	263	252
12	..	186	360	282
13	5	..	..	..
17	43	..	..	..
21	144	..	..	..
24	94	481	797	530
38	79	..	..	..
64	140	..	..	..

The tests at the different temperatures were not made simultaneously with the same spore suspension for inoculation, and for that reason the number of lesions resulting are not strictly comparable. The results show, however, that with low temperatures a longer subjection of the plants to high relative humidity is necessary for infection than at higher temperatures. The optimum temperature for infection appeared to be between 60° and 75° F. No infection was obtained where the foliage was dried within less than seven hours after inoculation and maintained dry thereafter.

The length of time that conidia of *D. rosae* are viable on the foliage after inoculation and drying is shown in table 8. Potted plants in the greenhouse were inoculated on the upper surface of the leaves on a day when the foliage would dry quickly. The plants were spaced apart from one another and an electric fan was used to facilitate drying. The temperature and the humidity were normal in the rose house for the rest of the experiment. Then, at intervals of days as shown, the foliage of a single plant was rewet with distilled water and the plant was placed in a chamber under conditions favorable for infection. The foliage was prevented from drying for a period of twenty-four hours, after which the plant was returned to the greenhouse. The results show an inverse relation between the length of time the plant was kept dry after inoculation, before being rewet, and the number of lesions that developed.

TABLE 8. DURATION OF INFECTIBILITY OF CONIDIA OF *DIPLOCARPON ROSAE* ON FOLIAGE AFTER INOCULATION AND DRYING

Length of time foliage was kept dry (days)	0	1	2	3	4	5	7	9	13	15	17
Foliage rewet. Amount of infection resulting											
Test A. Number of lesions per plant	...	469	262	242	26	73	..	..	..	..	..
Test B. Number of lesions per 100 leaflets	239	106	89	72	37	14	4	21	3	0	0

The period of liability of a plant to infection by conidia of *D. rosae* after inoculation depends probably on environmental conditions such as relative humidity and temperature. Subsequent tests have shown that a few conidia may remain viable for as long as three weeks after inoculation.

The relative humidity of the atmosphere required for conidial infection by the black-spot fungus was found to be above 92 per cent. The greatest amount of infection occurred when the foliage was kept wet for twenty-four hours after inoculation. A slight amount of infection occurred when the relative humidity around the plant was 96 per cent, which probably meant that the humidity close to the leaf surfaces was saturation or nearly so. There was only a slight difference in infectivity whether the upper or the lower surface of the leaf was inoculated. The relative humidity required in either case was about the same.

The relative humidity necessary for infection was determined by placing inoculated plants of the variety Pernet under bell glasses through which conditioned air was circulated. The desired relative humidity was obtained by drawing the air through saturated salt solutions as indicated by Shaw (1935). The procedure was as follows: Plants in pots were inoculated with a suspension of conidia and the foliage was then dried

as quickly as possible, this requiring from two to four hours. The pots were painted with paraffin to minimize evaporation and were placed under the bell glasses at room temperature, about 65° to 75° F., one plant for each chamber for the range of humidities used.

The air in each bell glass was changed at the rate of about 1 liter per minute. Wet- and dry-bulb thermometers and wet and dry thermocouples were tested as means of determining the relative humidities. Neither method as tested was satisfactory. Finally, single drops of saturated salt solutions such as were used to create the specific relative humidities, were placed on glass slides under the bell glasses. Evaporation of the comparative drops indicated air leaks and improper relative humidity. After forty-eight hours in the bell glasses, the plants were taken out, the paraffin was removed from the soil, and the plants were placed in the greenhouse until records of infection were obtained.

In each series of tests, a plant was placed under a closed bell glass with no air circulation. The air in this chamber was maintained at approximately 100 per cent relative humidity by wrapping wet absorbent paper around the pot. Check drops of water on glass slides remained without drying, as evidence of the saturated condition of the atmosphere.

The salt solutions used to create the different relative humidities are given in table 9 (taken from Hatfield 1931, Lange 1934, and Shaw 1935).

TABLE 9. SALT SOLUTIONS USED FOR CONTROL OF RELATIVE HUMIDITIES

Control solutions	Relative humidity* (per cent)
Distilled water, room temperature.....	99
CaSO ₄ .2H ₂ O, 50-per-cent saturated solution.....	98
CaSO ₄ .2H ₂ O, saturated solution.....	97
C ₂ H ₃ O ₄ .2H ₂ O, saturated solution.....	96
NaBrO ₃ , saturated solution.....	92
K ₂ CrO ₄ , saturated solution.....	88

*The relative-humidity values vary slightly with fluctuations in temperature.

In tables 10 and 11, data are presented to show the influence of relative humidity on infection by conidia of *D. rosae*. In table 10 the results are for plants of which the upper surface of the leaves was inoculated, while table 11 gives the results of inoculating the lower surface.

The data in table 12 illustrate how the relative humidity varies in a rose greenhouse throughout a year. In a house of roses of the varieties Pernet and Mrs. Franklin D. Roosevelt, a Friez hygrothermograph was placed in one of the beds between the rose plants at about the center of the Pernet section. The house measured 310 feet long by 50 feet wide. One half of the house contained 4736 one-year-old Pernet roses; the other half contained 4800 Mrs. Franklin D. Roosevelt plants of the same age. The recording machine was raised slightly above the surface of the soil in the bed by the board on which it rested, and was protected from the direct rays of the sun by a shade placed directly on the top of the instrument case.

The average relative humidity for the year was calculated from tabulations of the relative humidity every four hours as obtained from the continuous recordings of the instrument.

TABLE 10. RELATIVE HUMIDITY REQUIRED FOR CONIDIAL INFECTION, THE LEAVES BEING INOCULATED ON THE UPPER SURFACE

Test	Relative humidity (per cent)	Number of lesions	Number of leaflets	Number of lesions per leaflet
1. August 15, 1936	100*	324	53	6.11
	99	93	52	1.79
	98	64	52	1.23
	97	6	55	0.11
	96	1	46	0.02
Check (not inoculated)	100	0	58	0.00
2. August 26, 1936	100*	1,600	91	17.58
	99	464	39	11.90
	98	485	33	14.70
	97	263	42	6.26
	96	27	36	0.75
Check (not inoculated)	100	0	41	0.00
3. September 14, 1936	100*	202	27	7.48
	96	0	26	0.00
	92	0	24	0.00
	88	0	29	0.00
	100	0	28	0.00
4. September 17, 1936	100*	291	41	7.10
	96	15	45	0.33
	92	0	43	0.00
	88	0	30	0.00
	100	0	35	0.00

*The first plant in each test was placed under the bell glass without the foliage being dried immediately after inoculation. The foliage of the other plants was allowed to dry for two to four hours after inoculation before being subjected to the designated relative humidities.

TABLE 11. RELATIVE HUMIDITY REQUIRED FOR CONIDIAL INFECTION, THE LEAVES BEING INOCULATED ON THE LOWER SURFACE

Test	Relative humidity (per cent)	Number of lesions	Number of leaflets	Number of lesions per leaflet
1. August 18, 1936	100*	468	31	15.10
	99	532	31	17.16
	98	592	39	15.18
	97	427	24	17.79
	96	164	28	5.86
Check (not inoculated)	100	0	52	0.00
2. September 10, 1936	100*	286	40	7.15
	96	2	27	0.07
	92	0	31	0.00
	88	0	30	0.00
	100	0	32	0.00
3. September 19, 1936	100*	418	27	15.48
	96	33	32	1.03
	92	0	35	0.00
	88	0	31	0.00
	100	0	26	0.00
4. November 6, 1936	100*	99	35	2.83
	96	2	24	0.08
	92	0	25	0.00
	88	0	18	0.00
	100	0	21	0.00

*The first plant in each test was placed under the bell glass without the foliage being dried immediately after inoculation. The foliage of the other plants was allowed to dry for two to four hours after inoculation before being subjected to the designated relative humidities.

From the hygrothermograph, records were obtained also of the number of hours when the relative humidity was extremely high (about 90 per cent) for each four-weeks period of the year. These data indicate the

TABLE 12. RELATIVE HUMIDITY IN A HOUSE OF ROSES FROM MAY 27, 1935, TO MAY 24, 1936 (DATA FOR PERIODS OF FOUR WEEKS)

Four-weeks period	Per cent average relative humidity ± standard error	Number of hours relative humidity was above 90 per cent	Number of times relative humidity was above 90 per cent continuously for 6 hours or more
May 27-June 23.....	65.8±1.5	55	5
June 24-July 21.....	69.7±1.5	149	16
July 22-August 18.....	68.2±1.6	128	9
August 19-September 15...	75.4±1.5	159	9
September 16-October 13...	66.5±1.4	92	8
October 14-November 10...	74.5±1.1	114	7
November 11-December 8...	73.4±0.7	45	3
December 9-January 5.....	64.0±0.7	2	0
January 6-February 2.....	56.0±1.0	0	0
February 3-March 1.....	67.8±0.8	10	0
March 2-March 29.....	76.5±0.9	67	5
March 30-April 26.....	71.4±0.9	16	1
April 27-May 24.....	73.2±1.4	126	11
Average for year.....	69.42±0.33		
Total.....		963	74

times of the year when black-spot infection is most likely to occur in rose houses. The number of times during each four-weeks period when the relative humidity was above 90 per cent for six hours or longer, also is given in table 12. These data, however, do not show exactly the periods of possible infection, because the relative humidity close to the leaf surfaces would naturally be higher than the average air humidity away from the leaves, especially if there was little or no air circulation inside the greenhouse.

The temperature conditions within the greenhouse were controlled, so far as was possible, to have the day temperatures about 68° F. and the night temperatures about 63°. The plants were given a semiprune (only the older or the weaker canes removed) on June 19, 1935. The practice of syringing with water every seven to ten days was followed throughout the year until September 1935. After that, syringing was discontinued and red-spider mites were held in check by spraying with a Selocide-New-Evergreen material.³ Four applications of this spray were made between September 1935 and June 1936, the dates being October 10 and 23, 1935, and February 18 and 29, 1936. Regular practices were followed with respect to general care and treatment of the plants in other ways.

The average relative humidity, as shown in table 12, was low during December, January, and February. Even within this period there were five times when conditions were favorable in adjacent greenhouses for black-spot infection, new lesions being observed. The plants in these other houses were being syringed, however, which altered the conditions from those cited above for the house in which the hygrothermograph was located.

EFFECT OF CULTURAL METHODS

The syringing of roses with water is probably the most important factor in the occurrence of black-spot in greenhouses. It is done primarily to remove the red-spider mite from the foliage. Unfortunately

³This material is described on page 26.

it ensures widespread dissemination of the fungus spores, and creates conditions favorable for their germination with resulting infection. Periods in which infection took place were found usually to be those when cloudy weather or rains occurred during or immediately after syringing (table 13), the cloudiness and generally high relative humidity retarding the rate of drying of the foliage and thereby promoting germination of and infection by the conidia.

TABLE 13. DATA ON THE OCCURRENCE OF NEW BLACK-SPOT INFECTIONS UNDER GREENHOUSE CONDITIONS

Date when new lesions were observed	Date of last previous syringing, and weather following it
June 17, 1935	June 8—cloudy and rain 3 days
June 29, 1935	June 19—cloudy and rain 2 days
Aug. 20, 1935	Aug. 9—sun 2 days, cloudy and rain 1 day
Aug. 28, 1935	Aug. 21—sun 1 day, cloudy and rain 1 day
Sept. 11, 1935	Aug. 29—cloudy and rain 2 days
Nov. 2, 1935	Oct. 26—sun 1 day, cloudy and rain 1 day
Nov. 21, 1935	Nov. 9—sun 1 day, cloudy and rain 4 days
Nov. 23, 1935	Nov. 9—sun 1 day, cloudy and rain 4 days
Dec. 21, 1935	Dec. 12—cloudy 3 days, rain 1 day
Dec. 23, 1935	Dec. 12—cloudy 3 days, rain 1 day
Jan. 2, 1936	Dec. 23—cloudy 1 day, sun 2 days, rain 1 day
Jan. 14, 1936	Jan. 4—cloudy and rain 2 days
Jan. 15, 1936	Jan. 7—cloudy 2 days, rain 1 day
Jan. 15, 1936	Jan. 8—cloudy 1 day, rain 1 day

In addition to the data presented in table 13, the relation of relative humidity to black-spot infection during a period of four days following the syringing was studied. The records of relative humidity, however, were obtained from a hygrothermograph inside an adjoining rose house, and not in the houses where infection was noted (with the exception of that of June 8). These data on humidity, therefore, are only an indication of the conditions of humidity at the times when new infections were noted. The number of hours during the four days after each syringing when the recorded relative humidity was above 90 per cent, for each of the given dates of syringing, was as follows: June 8, 14 hours; June 19, 13+ hours; August 9, 17+ hours; August 21, 26 hours; August 29, 8 hours; October 26, 44 hours; November 9, 25 hours; December 12, 0 hours; December 23, 0 hours; January 4, 0 hours; January 7, 0 hours; January 8, 0 hours. This indicates that during weather which is generally cloudy following syringing, the humidity close to the leaf surfaces may be favorable for infection although the general greenhouse atmosphere may not necessarily be of extremely high relative humidity. Thus, cloudiness following syringing is a factor in judging periods favoring black-spot infection.

The time that elapsed between syringing and the noting of new lesions on the leaves varied, in the above observations, from seven to fourteen days. The average was about ten days. The wide variation was due in part to failure in some instances to make observations of the new infections on the days when the symptoms first appeared.

When new lesions appear on roses in a greenhouse, many are found to develop at the same time, indicating definite periods of infection such as are given in table 13.

In experimental tests the greatest number of infections occurred when the foliage remained wet continuously for twelve to twenty-four hours after inoculation. Intermittent wetting and drying for short periods of time did not favor infection, even when the foliage had been wet for a total of eighteen hours.

In a preliminary test of intermittent wetting of foliage after inoculation, six potted rose plants of the variety Pernet were inoculated and the foliage was kept wet for three hours (temperature 21.7° to 23.9° C.). On one plant, used as a check, the foliage was kept continuously wet for twenty-four hours. The foliage on the other five plants was dried immediately after the three-hours period, and was kept dry for the remainder of the twenty-four-hours period. The drying of the foliage was facilitated by spacing the plants and circulating the air with an electric fan. The foliage of the five plants was rewet for three hours; it was then dried, and was kept dry for the rest of the second period of twenty-four hours. This was repeated for six days until the foliage of the five plants had been intermittently wet for a total of eighteen hours. The amount of infection resulting for the five plants which received the intermittent wetting was 6 lesions; that for the one plant with the foliage continuously wet for twenty-four hours after inoculation was 360 lesions, thus showing a much greater liability to infection from continuous than from intermittent wetting.

CONTROL

ERADICATION

Frequent reference has been made, particularly in the older literature, to the removal and burning of black-spotted leaves. It has been recommended also that fallen leaves around infected plants should be gathered and burned. These control measures have been recommended mainly with respect to outdoor roses, the overwintering leaves of which are the most probable source of inoculum for primary infections in the spring.

Under greenhouse conditions such practices may give temporary improvement, but, unless the work is done very thoroughly, practically no benefit is to be expected. In the process of picking off diseased leaves, many healthy-appearing leaves having latent infections which are not yet visible may be overlooked. For this reason, successive pickings of black-spotted leaves would be required. At best, such an eradication program is too tedious for general use, and only with a small number of plants would hand-picking of diseased leaves be practical.

A test of hand-picking of black-spotted leaves was conducted in 1935 in a section of 4736 Pernet roses, on one half of which (four beds) the black-spotted leaves had been removed, the other half being left without such treatment to serve as a check. The usual practice of weekly syringing was continued, but care was exercised not to splash water from the check beds to the plants from which the diseased leaves were removed.

The time required for the first hand-picking of the black-spotted leaves (August 30 to September 8, 1934) was $35\frac{3}{4}$ hours for 2368 plants, or 15 man-hours of labor per 1000 plants. The second hand-picking (September 10 to 18) required 13 hours for the 2368 plants, or $5\frac{1}{2}$ man-hours per 1000 plants.

The average number of black-spotted leaflets remaining per plant on October 26 was 2.0 ± 0.6 for the hand-picked area and 42.4 ± 3.6 for the check area. On November 20, without further eradication work, the hand-picked area showed an average of 7.9 black-spotted leaflets per plant, and the check area 25.5.

Although a considerable amount of the inoculum was removed by picking off the diseased leaves, the two successive eradications did not give complete control, and the area in which the hand-picking was attempted retained enough inoculum to maintain the disease in that part.

Repetition of hand-picking of black-spotted leaves from 400 of the Pernet plants used in the above experiment showed that black-spot was not completely controlled even after the plants had been gone over carefully several times (table 14).

TABLE 14. CONTROL OF BLACK-SPOT BY REMOVING THE DISEASED LEAVES
(DATA FROM 400 PLANTS)

Hand-picking	Date	Number of black-spotted leaflets removed
3d time.....	November 20, 1934	104
4th time.....	November 26, 1934	48
5th time.....	December 3, 1934	39
6th time.....	December 21, 1934	32
7th time.....	January 2, 1935	13
8th time.....	January 30, 1935	73

There is a possibility that greater success in controlling black-spot by eradication of the diseased leaves would have been obtained if the plants had been examined at more frequent intervals, as every three or four days for a period of time. This would have helped to eliminate those leaves which had latent infections at previous examinations, before spores could be disseminated from them.

Tests to eradicate the pathogene by means of sprays to "kill out" the fungus in the leaves after infection had already set in, gave only negative results. Fungicidal sprays were applied on black-spotted leaves, and these leaves were tagged. Records were taken at the time of spraying, and again later to determine whether the lesions had increased in size. In most cases the black-spots showed enlargement, indicating that the fungus was still alive and active. Injury from the spray was noted in some of the tests. The result of one application of the fungicides is given in table 15.

PROTECTION

The application of protectants has been the most widely practiced means for controlling the black-spot disease. Among the different treatments, the most prominent have been spraying with bordeaux mixture and dusting with sulfur. Other recommendations have been made for sprays of ammoniacal copper carbonate, potassium permanganate, cuprous oxide, lime-sulfur, wettable sulfurs, Burgundy mixture, potassium sulfide, and other materials. In greenhouses the application of sulfur to steampipes to bring about its volatilization and resulting deposition on the foliage has been the most general, but not a satisfactory, method of

TABLE 15. EFFICIENCY OF FUNGICIDES IN DISINFECTING LEAVES AFFECTED WITH DIPLOCARPON ROSAE

Test	Treatment	Effect on foliage	Black-spots larger : no larger
Results 9 days after spraying			
1	Lime-sulfur 1:40 Alphasol OT* 1:300	No injury; slight discoloration	10:0
2	Magnetic-spray wettable sulfur† 1 lb. to 25 gal.	No injury; slight discoloration	10:0
3	Koppers flotation sulfur‡ 1 lb. to 25 gal.	No injury; slight discoloration	8:0
4	Koppers flotation sulfur‡ 1 lb. to 25 gal. Alphasol OT* 1:300	No injury; slight discoloration	8:0
5	Cuprous oxide§ 1 lb. to 100 gal. Lethane spreader§ 1:200	No injury; slight discoloration	10:0
6	Check—no spray		10:0
Results 14 days after spraying			
7	Lime-sulfur 1:10 Alphasol OT* 1:100	Burn and slight discoloration	8:1
8	Lime-sulfur 1:20 Alphasol OT* 1:200	Slight burn and discoloration	7:1
9	Lime-sulfur 1:30 Alphasol OT* 1:300	No injury; slight discoloration	8:0
10	Lime-sulfur 1:40 Alphasol OT* 1:400	No injury; slight discoloration	9:0
11	Potassium sulfide 1:100 Soap flakes** 1:400	No injury; slight discoloration	8:0
12	Potassium sulfide 1:200 Soap flakes** 1:400	No injury; slight discoloration	8:0
13	Potassium sulfide 1:300 Soap flakes** 1:400	No injury; slight discoloration	9:0
14	Potassium sulfide 1:400 Soap flakes** 1:400	No injury; slight discoloration	8:0
15	Check—no spray		8:0
16	Check—no spray		8:0

*Alphasol OT spreader, from American Cyanamid & Chemical Corporation, 535 Fifth Avenue, New York City.

†Magnetic-spray wettable sulfur, from National Sulfur Company, New York City.

‡Koppers flotation sulfur, from Koppers Products Company, Pittsburgh, Pennsylvania.

§Cuprous oxide and Lethane spreader, from Röhm & Haas, Philadelphia.

**Ivory soap flakes.

controlling black-spot. Other means for vaporizing sulfur in greenhouses have been advocated.

A greenhouse test using dusting sulfur for protection against black-spot was made with the Pernet variety of rose. Two sections in the same house were compared, one of which was given a light treatment regularly twice a week with dusting sulfur⁴ from June 8, 1935, to January 28, 1936, the other being maintained without a spray or a dust application. Both sections were syringed at about weekly intervals. After thirty-three weeks a count showed 8.4 ± 0.8 black-spotted leaflets per plant on

⁴The material used was Kolotex, a dusting sulfur containing 300-mesh sulfur, bentonite sulfur, and 10 per cent of arsenate of lead, manufactured by the Niagara Sprayer & Chemical Company, Middleport, New York.

the plants receiving the sulfur dust, and 17.8 ± 1.0 on the check plants which were not dusted. The sulfur dust thus gave a significant control of black-spot amounting to approximately 50 per cent.

The efficacy of dusting sulfur as a protectant against the black-spot disease was further demonstrated by three trials with potted Pernet plants which were artificially inoculated after a single dust treatment. The plants, immediately after being dusted, were atomized with a suspension of conidia of *Diplocarpon rosae* and placed in an inoculation chamber with moisture-saturated atmosphere for a period of twenty-four hours. Check plants which received no dusting sulfur were inoculated at the same time with the same spore suspension, and placed in the inoculation chamber with the dusted plants. Only the undusted (check) plants showed the development of black-spot to an appreciable extent (table 16).

TABLE 16. PROTECTANT ACTION OF ONE APPLICATION OF DUSTING SULFUR AGAINST ARTIFICIAL INOCULATION WITH CONIDIA OF DIPLOCARPON ROSAE

Date of application	Treatment	Number of lesions per 100 leaflets	Per cent control of black-spot
January 29, 1934	1 a. Check	780 ± 51	91
	1 b. Kolotex	69 ± 7	
May 18, 1934	2 a. Check	$1,595 \pm 282$	98
	2 b. Kolotex	35 ± 9	
June 1, 1934	3 a. Check	482 ± 92	90
	3 b. Kolotex	46 ± 9	

The use of fungicides either as sprays or as dusts, against black-spot of roses, has had certain disadvantages which have prevented their general use in the commercial greenhouse. The chief objection has been the discoloration of foliage and blossoms. This had been particularly true of sprays. With the advent of dusting sulfurs green in color, the discoloration was greatly reduced. Nevertheless there was still an unnatural appearance of the dusted leaves. Besides the unsightliness of the fungicides, many of the materials, particularly the copper sprays, caused injury to the leaves which was frequently as objectionable as black-spot.

Furthermore, results obtained in applying fungicides to roses growing under glass have not been generally satisfactory. The use of a fungicide as a protectant necessitates frequent application, to ensure coverage of the new growth. Spraying requires considerable time, labor, and apparatus. The use of dust protectants, although time-saving, has been found to be disagreeable for the workmen in that the dusts often cause irritation to eyes, nose, and throat.

These considerations have led to the testing of other means for combating the black-spot disease.

Protection by manipulation of the environment

Investigations were made of the possibility of circulating the air in the greenhouse so as to break up any zones of high relative humidity close around the plants. Twenty 10-inch oscillating electric fans were mounted along the two side walls of a house containing roses of the variety Pernet. The section measured 150 feet long by 22 feet wide. The longitudinal

direction of the house was east and west. The north side was of board construction to a height of about 6 feet. The fans were at first placed 16 feet apart and were later moved to 8 feet apart. The fanning did not move the air sufficiently to affect the layers of air close to the leaf surfaces, and little or no benefit was obtained.

The fanning did not adequately speed up or break up the natural air circulation, the direction of which when tested was a rising current along the south side to the ridge ventilator, then down along the north side of the house and back across the four beds. There was also a slight movement of air from one end of the house toward the opposite end, depending on the general wind direction. The average air flow in the walks and about 4 feet above the ground was estimated to be 0.43 ± 0.03 feet per second without using the fans, as determined from ammonium-chloride-fume movement (Fairbanks, 1928). With the fans located 8 feet apart to give a rotation of air lengthwise of the house, the air flow was 0.95 ± 0.05 feet per second.

That the fanning was inadequate or ineffective was proved by the fact that black-spot developed as much in the fanned section as in a corresponding area which had no artificial air circulation. Furthermore, records taken of the time to dry the foliage after syringing showed that the fans were not causing the leaves to dry any faster than where the fans were not used.

The ineffectiveness of the fanning may have been due to the tendency of the air movement to bring the temperature within the leaves to that of the air in the greenhouse. Any reduction of the leaf temperature would decrease the vapor-pressure gradient which determines the rate at which the water vapor diffuses from the leaf.

Sunlight, and increasing the amount of steam heat in the greenhouse, were found to be factors which contributed to the most rapid drying of the rose foliage after syringing.

Methods to obviate syringing

Syringing has been the general practice for control of the red-spider mite, an insect pest common to greenhouse roses. Because the syringing enhanced the likelihood of black-spot, methods to obviate this procedure were sought. On the advice of Dr. W. E. Blauvelt, entomologist at Cornell University, certain insecticidal sprays were tested as a means of making syringing unnecessary for the control of the red spider (Lyle and Massey, 1936).

Tests were first made with a pyrethrum-rotenone spray.⁵ In a greenhouse of roses of the variety Sweet Adeline, a section of 1440 plants was withheld from syringing from October 18 to December 5, 1934. The pyrethrum-rotenone spray at about 1:200 dilution was applied at a pressure of 50 pounds with a hand sprayer four times during the seven-weeks period. A check section of 2880 plants was given regular syringing and no spray.

On November 23, about five weeks after the test was started, the check plants which were syringed regularly without any spray showed 41.5 ± 2.2 black-spotted leaflets per plant. On the plants which received the

⁵The material used was Foliafume, produced by S. B. Penick & Company, 132 Nassau Street, New York City.

pyrethrum-rotenone spray and no syringing, there was an average of 8.4 ± 0.9 black-spotted leaflets per plant, showing approximately 80 per cent control of the disease in the unsyringed area. Three weeks later, on December 13, the syringed plants had 39.6 ± 2.2 black-spotted leaflets per plant, and the plants sprayed instead of syringed had 3.5 ± 0.6 . This is a reduction of about 91 per cent in the disease, brought about by the omission of syringing for a period of approximately seven weeks.

Although a good control of black-spot was obtained, the pyrethrum-rotenone spray under the conditions of its use did not give adequate control of the red spider.

Another test of omitting syringing and substituting the pyrethrum-rotenone spray was made with 1218 Pernet plants from September 20 to October 26, 1935. Three applications of the spray diluted 1:200 were made with a power sprayer at 300 pounds pressure, and care was taken to ensure thorough coverage of the foliage. In the section treated, the amount of infection was reduced from 19.1 ± 0.8 to 3.3 ± 0.3 black-spotted leaflets per plant in the five weeks, a significant reduction of about 83 per cent in the amount of black-spot. Here again, however, the pyrethrum-rotenone spray did not give adequate control of the red spider, and its use was discontinued.

An acaricide called *Selocide*⁶ also was tested as a substitute for syringing (tables 17 and 18). This material is a potassium-ammonium-seleno-sulfide (Gnadinger, 1933). It has been efficacious in controlling the red-spider mite on such plants as carnations, and has been used in the Pacific Coast area to combat the mites on citrus (Gnadinger, 1933; Boyce, 1936) and on grapes (Lamiman, 1933). This selenium spray material is said to be specific against the red-spider mite, and has not been demonstrated to be effective against other types of insects. It has been tested on a number of kinds of plants, and is recognized as likely to injure the foliage of certain plants, such as roses, when used at concentrations necessary for mite control.

Preliminary tests by the author showed that *Selocide* concentrations stronger than 1:500 are necessary for effective action against the red-spider mite on greenhouse roses. Concentrations of about 1:300 were found to be safe and effective.⁷ Dipping the rose foliage in solutions of the selenium compound was not suitable for control of the red spider, as was evidenced by tests with young potted rose plants. Using a hand sprayer at approximately 50 pounds pressure was not nearly so effective as spraying with a power machine at 300 pounds pressure.

Selocide used as dilute as 1:300 does not spread well on rose foliage, particularly on the younger growth. The addition of a pyrethrum-oil-soap product⁸ was found to be the best method tested to improve the spread and action of *Selocide*. This material proved superior to different plain liquid soaps which were tried. Under the conditions in the greenhouses at Rowayton, Connecticut, the best combination of spray was

⁶*Selocide* is manufactured by the McLaughlin Gormley King Company, Minneapolis, Minnesota. The form used was said to contain an 8-per-cent solution of the selenium material. A more concentrated form is available, but was not tested.

⁷Since the completion of these experiments, numerous reports have been received of failure to get satisfactory control of the red-spider mite with *Selocide*, and of decreasing efficiency with its continued use even at greater concentrations. It is hoped that experiments under way at this Station and elsewhere may supply the solution to this problem.

⁸The material used was New Evergreen, a pyrethrum-oil-soap material manufactured by the McLaughlin Gormley King Company, Minneapolis, Minnesota.

found to be Selocide 1:300 and the pyrethrum-oil-soap added at 1:1000. It is suggested here that the proper concentrations to be used vary with local conditions of water hardness—the harder the water, the stronger the spray solution that should be used.

TABLE 17. CONTROL OF BLACK-SPOT THROUGH THE OMISSION OF SYRINGING, WITH USE OF SELOCIDE SPRAY FOR RED-SPIDER-MITE CONTROL. NO UNSPRAYED AREAS FOR CHECKS

Test	Variety, date when test was started, and number of plants	Dates of records	Number of black-spotted leaflets per plant
1	Pernet September 20, 1935 1,218 plants	September 23, 1935 October 26, 1935 November 29, 1935 February 6, 1936	19.1 $\pm$ 0.8 3.3 $\pm$ 0.4 0.73 $\pm$ 0.14 0.04 $\pm$ 0.03
	(99.8 per cent reduction of black-spot in 136 days)		
2	Premier Supreme December 20, 1935 10,000 plants	December 18, 1935 February 17, 1936 March 25, 1936 April 28, 1936	24.8 $\pm$ 0.8 1.7 $\pm$ 0.2 0.37 $\pm$ 0.07 0.17 $\pm$ 0.01
	(99.3 per cent reduction of black-spot in 132 days)		
3	Sweet Adeline December 19, 1935 10,000 plants	December 19, 1935 January 27, 1936 February 25, 1936 March 25, 1936 April 27, 1936	19.0 $\pm$ 0.6 13.8 $\pm$ 0.5 1.3 $\pm$ 0.1 0.06 $\pm$ 0.02 0.008 $\pm$ 0.008
	(99.9 per cent reduction of black-spot in 130 days)		
4	Rapture February 11, 1936 2,376 plants	February 13, 1936 March 27, 1936 April 29, 1936	14.6 $\pm$ 0.8 2.7 $\pm$ 0.36 0.63 $\pm$ 0.14
	(95.7 per cent reduction of black-spot in 76 days)		
5	Briarcliff February 2, 1936 10,000 plants	January 29, 1936 March 3, 1936 April 3, 1936 April 28, 1936	23.2 $\pm$ 0.9 2.3 $\pm$ 0.2 0.9 $\pm$ 0.1 0.47 $\pm$ 0.07
	(98.0 per cent reduction of black-spot in 90 days)		

TABLE 18. CONTROL OF BLACK-SPOT WITH SELOCIDE SPRAY SUBSTITUTED FOR SYRINGING. CHECK AREAS MAINTAINED WITH SYRINGING AND NO SPRAYING

Test	Variety, date when test was started, and number of plants	Number of black-spotted leaflets per plant			
		Dates of records	Selocide-sprayed	Checks	Difference
1	Premier Supreme November 9, 1935 9,640 plants	December 12, 1935	8.2 $\pm$ 0.4	24.8 $\pm$ 0.9	16.6 $\pm$ 1.0
	(66.9 per cent reduction of black-spot in 33 days)				
		February 11, 1936	1.4 $\pm$ 0.2	18.3 $\pm$ 0.8	16.9 $\pm$ 0.8
	(92.3 per cent reduction of black-spot in 94 days)				
2	Rapture December 3, 1935 7,128 plants	February 13, 1936	2.0 $\pm$ 0.7	14.6 $\pm$ 0.8	12.6 $\pm$ 1.1
	(86.3 per cent reduction of black-spot in 72 days)				
3	Pernet September 20, 1935 3,654 plants	February 7, 1936	0.04 $\pm$ 0.03	17.8 $\pm$ 1.0	17.8 $\pm$ 1.0
	(99.8 per cent reduction of black-spot in 140 days)				

The effective control of the black-spot disease of roses where syringing was replaced by Selocide spray, is shown in tables 17 and 18.

The tests cited above show conclusively that black-spot can be satisfactorily controlled by withholding syringing for a time. In the comparative experiments in which check areas were maintained, the sprayed and the syringed areas for each variety were in the same house and adjacent to each other except in the case of the variety Pernet. Syringing was done so as not to carry water over onto the Selocide-treated part. With the variety Pernet, the check plants and the sprayed plants were in different houses, but they were of the same age and had received similar treatment previous to the test. The Pernet roses, as also the other varieties, had comparable amounts of black-spot in the sprayed and the syringed areas at the beginning of the trial. The syringed, or check, plants were syringed on an average of once every seven to ten days.

Records were compiled on the expense of spraying with Selocide, based on the winter period when the experiments were conducted. It was found that the cost of spraying was not more than twice that for syringing, considering only the labor and the material and not the reduction in black-spot and other benefits where syringing was omitted. The labor expense of syringing was estimated to be about \$10.80 per 1000 plants per annum where syringing was done once every two days, one man syringing 1000 plants an hour with the labor cost at 30 cents an hour. This does not include the cost of excessive tying-up of rose plants where syringing is done, nor does it include the damage from disease, the tearing of foliage, and the crooked stems which result from syringing.

The cost of spraying with Selocide 1:300 and New Evergreen 1:1000 was estimated at about \$11 per 1000 plants per annum for spray materials and about \$7.20 per 1000 plants per annum for the labor involved. These figures were based on several months testing where the amount of spray used was 1 gallon of solution to fifteen plants, with twelve applications a year. The labor was figured at 30 cents an hour. The cost of the power sprayer equipment and its upkeep were not included in these estimates.

Some consideration has been given to the claim that water on the foliage, such as is acquired from syringing, is a benefit to plant growth and flower production. In one house of Pernet plants and in two of Francis Scott Key, the foliage was sprinkled with water each sunny day for a period of six weeks (April 4 to May 16, 1936). No benefit was seen from having water on the foliage during that time, in comparison with plants of the same varieties in houses where the foliage was kept dry. If there is a single disadvantage or harmful effect from the omission of syringing, it has not been observed.

With regard to the use of Selocide, certain precautions have been found necessary. The most likely source of plant injury when using concentrations of 1:300 or higher is from combination with vaporized sulfur from steampipes. A period of about a week should elapse between the use of Selocide and volatilizing sulfur from steampipes, or from having sulfur on the steampipes and spraying with Selocide. Certain investigations have shown that, although vaporized sulfur is not compatible with Selocide spray, dusting sulfur may be used instead of vaporized sulfur

without serious injury resulting. This refers to the control of rose mildew, which may develop during the time when Selocide spraying is being done.

The addition of a wettable sulfur to Selocide has been tested (W. E. Blauvelt, data unpublished), and has been found to increase the effectiveness of Selocide in red-spider-mite control; but the question of its relative safety remains to be determined.

The matter of timing the sprays has been found important in respect to red-spider-mite control. The best results have been obtained by applying two or three successive sprays at weekly or ten-days intervals and then withholding further spraying until it is again found necessary (which in some cases has been three or four months). This has been preferred to spraying at monthly intervals or by some other arbitrary schedule. Selocide at dilute concentrations is not very effective against the egg stage of the red-spider mite, and successive sprays at weekly or ten-days intervals has been found to give control of the adult forms of the red spider before further reproduction occurs. Warm weather may shorten somewhat the interval between sprays, owing to the increase in rate of transition of the mite from one stage to another.

Selocide spray may react with lead-painted surfaces in greenhouses to cause discoloration. The paint is turned to a brownish or blackish color. The spray does not affect aluminum paints.

There has been some claim of selenium-poisoning which warrants precaution. Gnadinger's report (1933) on selenium as an insecticidal material discusses the likelihood of selenium toxicity to plants from accumulations in the soil. It also points out the poisonous characteristic of certain selenites when given internally to animals. The effect of the form and the amount of selenium which occurs in Selocide spray has not been fully determined.

The use of some material for mite control other than Selocide would doubtless serve as effectively for black-spot control, since the factor involved is that of keeping the foliage dry through the omission of syringing.

IMMUNIZATION

The development in rose plants of resistance to black-spot by the addition of extra amounts of fertilizer and manure was mentioned in a report by Dodge (1932). Gregory (1931) reports that many growers in Indiana believe that keeping the plants growing vigorously and steadily by the liberal use of fertilizers and copious watering is an important factor in black-spot control. A simple test of such treatment by the writer gave no indication of its being effective in lessening the amount of disease on the plants receiving the treatment. In a house of roses of the variety Pernet, one half of a bed was given additional manure and fertilizer. Two extra applications of cow manure and one of 10-20-20 fertilizer were made in eight weeks time. The cow manure was applied as a light dressing, and the fertilizer was put on at the rate of about 0.7 pounds to 100 square feet. The rest of the house was given neither manure nor fertilizer until after a record of the amount of black-spot was taken. The entire house was syringed regularly during the period of the test.

On February 7, 1936, the bed receiving the extra manure and fertilizer

had 21.8 ± 1.9 black-spotted leaflets per plant. The three check beds without extra manure or fertilizer had, respectively, 16.4 ± 2.0 , 17.4 ± 2.4 , and 21.4 ± 2.7 black-spotted leaflets per plant. No difference between any two of the results is significant. The additional manure and fertilizer neither stimulated nor reduced the incidence of black-spot.

SUMMARY

Black-spot of roses, caused by the fungus *Diplocarpon rosae* Wolf, is a serious disease in most commercial rose houses. The object of the experiments recorded here was to discover additional facts in the development of the pathogene and to find more effective means of control.

The disease was found to attack all the varieties of greenhouse roses tested, provided the conditions favored its occurrence. Certain varieties, however, are more affected than others in the commercial range. Premature defoliation from black-spot may substantially reduce the number of leaves per plant, as much as 20 per cent difference being observed between healthy and diseased individuals.

The symptoms of black-spot are black necrotic areas, mainly on the leaves but occurring also on the sepals, receptacles, and flower stems, and on the canes themselves. Lesions on the leaf blades are evidenced usually on the upper surface only if infection originates there, and on both sides when infection takes place on the lower surface. The signs of the disease are the acervuli and spore mounds in the necrotic spots.

Only secondary cycles of the fungus are common in greenhouses, the pathogene being either carried over from one season to the next in leaves which are left during pruning, or brought into the houses in infected plants during replanting. The abundance of inoculum for spread of the pathogene is demonstrated from an estimate of an average of 32,000 conidia per leaf spot 6.3 millimeters in diameter (data from outdoor roses). Dissemination of the spores is accomplished mainly by splashing water.

Tests indicate that prewetting of the conidia is necessary before germination will occur, and contact with water or an extremely high relative humidity is a prerequisite for the development of the spores to produce leaf infection. Temperature is not an important factor in the occurrence of black-spot in the greenhouse, except as it affects relative humidity.

Syringing of roses with water in combating the red-spider mite is given as the principal reason for the spread and severity of black-spot under glass. Methods to obviate syringing are shown to be the most effective means of controlling the disease, pyrethrum-rotenone or selenium-containing spray being substituted for syringing. The latter was found to be the more satisfactory material.

Application of a fungicidal protectant, such as dusting sulfur, proved to be partially effective in preventing black-spot. Other tests to control the disease by hand-picking the infected leaves, or by circulating the air by means of electric fans to disperse any high relative humidity around the leaves, favoring infection, did not solve the problem, nor did they accomplish their purposes. An experiment testing the effect of additional manure and fertilizer showed that no change resulted in the resistance of the plants to black-spot.

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